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## **Cis interactions of immunoreceptors with MHC and non-MHC ligands**

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### **Abstract**

The conventional wisdom is that cell-surface receptors interact with ligands expressed on other cells to mediate cell-to-cell communication (*trans* interactions). Unexpectedly, it has recently been found that two classes of receptors specific for MHC class I molecules not only interact with MHC class I molecules expressed on opposing cells, but also with those on the same cell. These *cis* interactions are a feature of immunoreceptors that inhibit, rather than activate, cellular functions. Here, we review situations in which *cis* interactions have been observed, the characteristics of receptors that bind in *trans* and *cis*, and the biological roles of *cis* recognition.

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As their name indicates, MHC molecules were discovered and characterized based on their role in inducing potent rejection of tissue grafts. However, the physiological role of MHC molecules remained enigmatic until ground-breaking studies demonstrated the fundamental importance of the MHC complex in T-cell-mediated immunity to infection<sup>1</sup>. A vast amount of subsequent work elucidated how T-cell receptors (TCRs) recognize self MHC molecules in complex with non-self peptides derived from microbial or other foreign proteins.

Transplantation biology provided early evidence for the existence of an alternative immune-recognition strategy. The laws of transplantation predicted that tissue grafts were accepted as long they displayed a subset of the recipient's MHC alleles<sup>2</sup>. Indeed, MHC heterozygous offspring from two mouse strains that differed at the MHC locus (F1 hybrids) accepted solid tissue grafts from either parent. However, parental bone-marrow grafts were rejected by irradiated F1 hybrid mice, a phenomenon termed hybrid resistance<sup>3</sup>. This observation suggested a novel type of immune recognition, which was controlled by MHC genes. The basis for this phenomenon remained obscure until several lines of research converged on the proposal that the lack of (appropriate) MHC class I molecules on host cells resulted in natural killer (NK)-cell-mediated rejection (the 'missing-self' hypothesis)<sup>4</sup>. Subsequent work showed that NK cells expressed MHC-class-I-specific inhibitory receptors, which protected normal host cells from NK-cell-mediated attack. Consequently, the loss of MHC class I molecules, which arises owing to infection or transformation, and is likely selected for by cytolytic T cells, renders host cells susceptible to NK-cell-mediated lysis, as inhibitory receptors are no longer engaged. These studies led to the identification of families of MHC class I receptors expressed by NK cells. Hybrid resistance was eventually explained by the selectivity of inhibitory receptors for certain MHC class I alleles together with the differential expression of these receptors by subsets of NK cells. As a consequence, a subset of NK cells in MHC heterozygous

hosts may fail to engage parental MHC class I molecules<sup>5</sup>, which results in NK-cell-mediated rejection of bone-marrow grafts.

#### **'Missing-self' hypothesis**

The concept that absence of MHC class I expression renders host cells sensitive to lysis by natural killer cells.

Inhibitory NK-cell receptors specific for classical MHC class I molecules belong to the mouse Ly49 (also known as KLRA) receptor family<sup>6,7</sup> and the human killer immunoglobulin-like receptor (KIR) family<sup>8-10</sup>. The molecular cloning of NK-cell receptors led to the identification of additional MHC class I receptors, which had not been predicted on the basis of functional experiments: human leukocyte immunoglobulin-like receptors (LILRs; also known as LIRs, ILTs or MIRs), and their orthologues, mouse paired immunoglobulin-like receptors (PIRs), as well as the heterodimeric CD94-NKG2 (NK group 2) receptors<sup>11-14</sup> (TABLE 1).

All of these MHC class I receptors were identified based on their capacity to regulate immune-cell function on binding in *trans* to MHC ligands expressed by other cells (antigen-presenting cells (APCs) in the case of TCRs, and target cells in the case of NK-cell receptors). Recently, however, the structurally unrelated NK-cell receptors Ly49 and LILRs or PIRs were found to interact with MHC class I ligands expressed in the plane of the same membrane (*in cis*)<sup>15, 16</sup>. Here, we examine the possible structural basis for *cis* versus *trans* binding of MHC molecules by these receptors and discuss the physiological relevance of *cis* and *trans* interactions for immune-cell function. We further review evidence that some receptors that recognize non-MHC ligands also act both in *cis* and *trans*, indicating that *cis* interactions may need to be considered alongside conventional *trans* interactions in the functional analysis of certain immunoreceptors.

## **MHC recognition in *trans* and *cis***

### **T-cell receptors**

X-ray crystallographic studies of TCRs bound to peptide-MHC-class-I or peptide-MHC-class-II complexes have shown that the TCR is typically positioned diagonally across the composite surface created by the peptide and the MHC  $\alpha$ -helices that flank the peptide-binding groove, with the variable- $\alpha$  ( $v\alpha$ ) domain situated over the N-terminal half of the peptide and the  $v\beta$  domain over the C-terminal half<sup>17</sup> (FIG. 1a). The affinity of this interaction determines the fate of T cells during thymocyte development and the type of immune response that T cells will elicit when mature. These effects are mediated by *trans* recognition of peptide-MHC complexes expressed by stromal cells or APCs. Although T cells express MHC class I molecules, this expression is not required for thymocyte positive selection<sup>18</sup> or for the activation of peripheral T cells<sup>19</sup>. Thus, there is no evidence that TCRs functionally interact with MHC molecules in *cis*. Indeed, structural considerations would exclude this possibility, as the segments connecting both TCR and MHC molecules to their respective transmembrane domains appear too short (10-20 residues) to permit a TCR to engage an MHC molecule on the same cell. Moreover, segmental flexibility between the  $v\alpha v\beta$  and  $C\alpha C\beta$  modules of the TCR, as well as between the  $\alpha 1$ ,  $\alpha 2$  and  $\alpha 3$  domains of an MHC class I molecule, is too restricted to compensate for the shortness of the connecting peptides.

#### **Positive selection**

One step in the process of T-cell differentiation in the thymus. Thymocytes expressing T-cell receptors with moderate affinity for self-peptide-MHC complexes receive a survival signal and continue to develop towards becoming single positive (CD4<sup>+</sup>CD8<sup>-</sup> or

CD4-CD8<sup>+</sup>) T cells. Positive selection is mediated by resident stromal cells in the thymic cortex.

### Ly49 receptors

Ly49 receptors are homodimeric type II membrane proteins, with each chain composed of a C-type lectin-like domain (CTLTD) connected by a stalk region of approximately 70 amino-acid residues to transmembrane and cytoplasmic domains. The Ly49 receptor family comprises at least 23 members in mice (Ly49A-Ly49w), 13 of which have an inhibitory function and 10 of which have an activating function<sup>20</sup>. This type of receptor is expressed by rodent but not human NK cells, which instead express KIRs to monitor MHC class I expression. Different Ly49 receptors exhibit distinct MHC-binding properties, ranging from the broad recognition of MHC class I molecules by Ly49C to the allelic specificity of Ly49A<sup>21</sup>. Bound peptide is necessary for MHC class I recognition by Ly49 receptors, although binding to Ly49A is independent of the peptide sequence<sup>22</sup>. Conversely, the Ly49C and Ly49I receptors are peptide selective, despite the absence of direct contacts with peptide<sup>23-26</sup>.

#### Type II membrane proteins

An integral membrane protein, such as Ly49, in which the carboxy terminus is extracellular.

#### C-type lectin-like domain

(CTLTD). A protein module originally identified as a carbohydrate-recognition domain in a family of calcium-dependent lectins. The natural-killer-cell receptor group of C-type lectin-like receptors includes disulphide-linked homodimers or heterodimers that do not bind calcium and recognize proteins instead of carbohydrates.

In contrast to TCRs, which dock onto the top of MHC molecules, structural and mutagenesis studies of complexes of Ly49A with H2-D<sup>d</sup>, and of Ly49C with H2-K<sup>b</sup>, revealed that Ly49 receptors contact MHC class I molecules at a wide cavity underneath the peptide-binding platform<sup>26-28</sup>. This binding site, which partially overlaps that of CD8 (REF. 29), is formed by the  $\alpha 2$  and  $\alpha 3$  domains of MHC class I and  $\beta_2$ -microglobulin ( $\beta_2m$ ). In the Ly49C-H2-K<sup>b</sup> complex<sup>26</sup>, the Ly49C homodimer engages H2-K<sup>b</sup> bivalently, such that each CTLTD makes identical interactions with MHC class I to form a symmetrical, butterfly-shaped assembly (FIG. 1b). By contrast, in the Ly49A-H2-D<sup>d</sup> complex<sup>28</sup>, the Ly49A dimer contacts H2-D<sup>d</sup> asymmetrically, with only one of its subunits binding an MHC molecule (FIG. 1c). A second potential MHC-binding site, originally observed in the Ly49A-H2-D<sup>d</sup> crystal structure<sup>27</sup>, was ruled out as not functionally relevant by subsequent mutagenesis studies<sup>30,31</sup>. The structural basis for the very different modes of MHC engagement in the Ly49C-H2-K<sup>b</sup> and Ly49A-H2-D<sup>d</sup> complexes is dependent on the different geometries of the Ly49C and Ly49A dimers. The Ly49C dimer adopts an 'open' conformation, in which the CTLTD subunits are less closely juxtaposed than in the Ly49A dimer, which adopts a 'closed' conformation (FIG. 1b,c). Important steric clashes between MHC molecules would preclude the closed Ly49A dimer from simultaneously binding two MHC in the manner of the open Ly49C dimer. However, a nuclear magnetic resonance (NMR) study of unbound Ly49A revealed that the receptor exists predominantly in the open state in solution, similar to Ly49C, and that the open form of Ly49A can bind two MHC molecules<sup>32</sup>. Hence, Ly49A (and presumably other Ly49 receptors) can assume both open and closed conformations, resulting in differential engagement of MHC ligands that might correlate with *trans* and *cis* binding, respectively (see later).

The capacity of Ly49A to bind H2-D<sup>d</sup> on other cells differs markedly between NK cells from mice that express H2-D<sup>d</sup> and mice that lack H2-D<sup>d</sup> expression<sup>33</sup>. This effect is due to the expression of H2-D<sup>d</sup> on the NK cells themselves, which masks Ly49A and reduces its capacity to bind H2-D<sup>d</sup> ligand on other cells<sup>15</sup>. Indeed H2-D<sup>d</sup> occupies at least 75% of the Ly49A molecules expressed by individual NK cells, reducing the accessibility of Ly49A by approximately fourfold<sup>34,35</sup>. Similar to Ly49A, most other Ly49 receptors of known MHC specificity have the capacity to interact with MHC class I molecules in *cis*<sup>25,36</sup>. The notable exception is the more distantly related receptor Ly49B, which only binds MHC class I molecules in *trans*<sup>25</sup>.

The inhibitory interaction of Ly49A with H2-D<sup>d</sup> on target cells (*trans* interaction) uses a binding site beneath the peptide-binding platform of H2-D<sup>d</sup> (REFs 27,30,31). This same binding site mediates the interaction with H2-D<sup>d</sup> in *cis*<sup>15</sup>. Consequently, Ly49 receptors must drastically reorient their CTLDs relative to the NK-cell surface to bind MHC class I in *trans* versus *cis* (FIG. 2a,b). It is most likely that the exceptionally long stalk regions linking the CTLDs of Ly49 molecules to the membrane provide the requisite flexibility. Although these proline-rich stalks have not been visualized in any Ly49 structure, they are predicted to consist of coiled-coil regions inter-spersed with several flexible segments that confer mobility to the ectodomains<sup>27</sup>. Based on the structure of the Ly49C-H2-K<sup>b</sup> complex<sup>26</sup>, the stalks must adopt a back-folded, or bent, conformation for the Ly49 dimer to bind in *trans*, as the N-termini of the CTLDs point away from the NK-cell membrane (FIG. 2a). Conversely, *cis* binding would require the stalks to assume an extended conformation that orients the CTLDs with their N-termini directed towards the NK cell (FIG. 2b). Although it is unknown whether one Ly49 dimer can bind two MHC class I molecules in *cis*, simple modelling suggests that engagement of both CTLDs by MHC molecules on the NK cell is unlikely, owing to the orientation that binding of one MHC molecule would impose on the Ly49 dimer (FIG. 2b). If so, the (bivalent) Ly49C-H2-K<sup>b</sup> and (monovalent) Ly49A-H2-D<sup>d</sup> complexes would exemplify *trans* and *cis* recognition, respectively.

## LILRs and PIRs

After the C-type lectin-like Ly49 receptors, the immunoglobulin-like LILRs and PIRs were the second class of immunoreceptors that were found to interact with MHC class I in *cis*<sup>16</sup>. As LILRs and PIRs also recognize MHC class I in *trans*<sup>13</sup>, structurally unrelated receptors have evolved corresponding capacities to bind MHC ligands expressed on the same membrane, as well as on opposing membranes, indicating convergent evolution. This suggests that *cis* recognition of MHC class I may be of general importance as a mechanism for regulating immune-cell function (see later).

The human LILRs comprise at least five inhibitory receptors (LILRB1-LILRB5) and three activating receptors (LILRA1-LILRA3), which are characterized by two or four extracellular immunoglobulin-like domains<sup>13,37</sup>. The homologous mouse PIRs, which contain six immunoglobulin-like domains, include a single inhibitory receptor (PIRB). At least six genes encode activating receptors (PIRA)<sup>14,38</sup>. LILRB1 is broadly distributed, whereas LILRB2 and PIRB are more selectively expressed by B cells and myeloid cell types, but not by T cells and NK cells. These receptors bind a wide range of classical and non-classical MHC class I molecules with low affinity and without any apparent allelic specificity (reviewed in REF. 39).

Structural knowledge of LILRs and PIRs is based mainly on studies of LILRB1 (REF. 40). MHC class I binding is mediated by the two most membrane-distal immunoglobulin-like domains (D1 and D2) of this four immunoglobulin-like domain receptor. The structure of LILRB1 (D1 and D2 only) in complex with HLA-A2 showed that LILRB1 binds at a site formed by the  $\alpha$ 3 domain of HLA-A2 and  $\beta$ <sub>2m</sub>, such that the tip of D1 contacts the  $\alpha$ 3 domain

and the D1-D2 interdomain hinge region contacts  $\beta_2m$  (FIG. 1d). A significant contribution by  $\beta_2m$ , which was also observed for PIRB<sup>41</sup>, helps to explain the broad MHC reactivity of LILRs and PIRs. Although the structure of the complete LILRB1 ectodomain (D1-D4) has not been determined, modelling indicates that the four immunoglobulin-like domains must adopt an extended conformation, similar to that reported for CD4 (REF. 42), to bind MHC class I on an opposing cell surface (FIG. 2c).

A similarly broad MHC recognition profile in *cis* and *trans*<sup>16</sup> provides circumstantial evidence that the same binding site of LILRB2 mediates both interactions. If so, *cis* recognition would require D1 and D2 to reverse direction with respect to the effector-cell surface, resulting in a horseshoe-shaped configuration of D1-D4 in which LILRB1 bends back on itself (FIG. 2d). Although such a large reversal implies considerable flexibility in the peptide segment connecting D2 and D3, the structure of the N-terminal four immunoglobulin-like domains of the *Drosophila melanogaster* protein DSCAM (Down syndrome cell-adhesion molecule) showed a horseshoe arrangement, which is made possible by a five-residue hinge between the D2 and D3 immunoglobulin-like domains of DSCAM, that is very similar to that proposed here for *cis*-binding LILRB2 (REF. 43). In the case of PIRB, which has two more immunoglobulin-like domains proximal to the membrane compared with LILRB2, additional flexibility might derive from the D4-D5 or D5-D6 connecting segments. Thus, Ly49 receptors and LILRs or PIRs appear to have evolved two distinct strategies to achieve *cis* and *trans* MHC recognition: unusually long stalk regions in the case of Ly49 receptors, and very flexible (or multiple) interdomain hinges in the case of LILRs and PIRs.

### KIRs and CD94-NKG2

Human NK cells use KIRs to monitor MHC class I expression on target cells (reviewed in REF. 44). These receptors, the role of which corresponds to that of Ly49 receptors in rodents, comprise two or three extracellular immunoglobulin-like domains, and hence are designated KIR2D and KIR3D, respectively. In contrast to both Ly49 receptors and LILRs, KIRs dock onto the top of an MHC class I molecule in a manner that resembles that of TCR binding<sup>45, 46</sup>. There is currently no evidence that the function of KIR2DL is influenced by HLA-C expression in *cis* (N. Gardiol and W.H., unpublished observations). KIR3D contains an additional N-terminal immunoglobulin-like domain (D0), which enhances MHC class I binding by the D1 and D2 domains<sup>47</sup>. A possible effect of a *cis* ligand on KIR3DL function has not been tested.

The heterodimeric C-type lectin-like CD94-NKG2 receptors, which are expressed on NK cells and some effector T cells, bind the non-classical human HLA-E or mouse Qa-1<sup>b</sup> molecules<sup>11,12</sup>. Structural models based on the complex between NKG2D (NK group 2, member D) and one of its ligands, MICA (MHC-class-I-polypeptide-related sequence A)<sup>48</sup>, together with the structure of the CD94-NKG2 heterodimer<sup>49</sup> and the role of specific residues in HLA-e and its bound peptide on recognition<sup>49,50</sup>, suggest that CD94-NKG2 binds to the  $\alpha 1$ - $\alpha 2$  platform domain of MHC class I, as do KIRs. Ligand binding by CD94-NKG2A is not influenced by Qa-1<sup>b</sup> expression by NK cells (L. Scarpellino and W.H., unpublished observations), suggesting that the inhibitory CD94-NKG2A receptor is not masked by *cis* ligands.

#### HLA-E

A non-classical MHC class I molecule with limited sequence variability. Its expression on the cell surface depends on the availability of peptides derived from the signal sequence of classical MHC class I molecules. HLA-E is recognized by CD94-NKG2 receptors.

**Qa-1<sup>b</sup>**

A functional mouse homologue of human HLA-E. Similar to HLA-E, Qa-1<sup>b</sup> cell-surface expression depends on the binding of peptides derived from the signal sequence of classical MHC class I molecules and it is recognized by CD94-NKG2 receptors.

Although there is at present no evidence for *cis* recognition by inhibitory receptors that bind to the top of MHC class I ligands (KIRs, CD94-NKG2), an unusual feature initially described for the non-classical MHC molecule haemochromatosis protein (HFE) raises the formal possibility of *cis* interactions for at least some of these receptors. HFE contributes to the maintenance of iron homeostasis by binding to the transferrin receptor (TfR)<sup>51</sup>. The structure of the complex between HFE and TfR indicates that these molecules associate in *cis* on the same cell membrane, rather than in *trans* between opposing cell membranes<sup>52</sup>. To do so, HFE 'lies down' parallel to the cell membrane, to allow the HFE  $\alpha$ -helices that form the peptide-binding groove in classical MHC class I molecules to contact TfR (FIG. 1e). Interestingly, HFE is recognized by  $\alpha\beta$  TCRs<sup>53</sup>, implying that it can also adopt an 'upright' configuration. If classical MHC molecules can assume a lying down orientation under certain circumstances, as has been suggested<sup>54</sup>, this may allow *cis* binding by immunoreceptors such as KIRs that engage MHC class I ligands via their  $\alpha 1$ - $\alpha 2$  platform domain. Irrespective of this possibility, however, the structure of the HFE-TfR complex<sup>52</sup> suggests that *cis* association may predate the immunological functions of MHC molecules.

**Haemochromatosis protein**

(HFE). A non-classical MHC class I molecule that regulates iron metabolism by binding to the transferrin receptor. The *HFE* gene is mutated in hereditary haemochromatosis — an iron overload disease.

**Role of MHC class I recognition in *cis***

To date, *cis* interactions have been documented exclusively for MHC class I receptors with inhibitory function. Such receptors are characterized by the presence of immunoreceptor tyrosine-based inhibitory motifs (ITIMs) in their cytoplasmic domain. When phosphorylated by SRC family tyrosine kinases, such as LCK or LYN, ITIMs recruit phosphatases, such as SHP1 (SRC-homology 2 (SH2)-domain-containing protein tyrosine phosphatase 1), that subsequently suppress phosphorylation-based activation signalling (reviewed in REF. 55). Importantly, efficient ITIM phosphorylation, and consequently inhibitory signalling, requires co-ligation of inhibitory receptor(s) with activating receptor(s)<sup>56</sup>. Accordingly, the role of MHC class I recognition by inhibitory receptors in *cis* needs to be considered in the context of the activation receptor(s) or pathways that are co-engaged.

**Immunoreceptor tyrosine-based inhibitory motif**

(ITIM). A short amino-acid sequence (the consensus sequence of which is Val/Ile-X-Tyr-X-X-Val/Leu, where X denotes any amino acid) that is found in the cytoplasmic tail of inhibitory receptors. ITIMs are thought to mediate inhibitory signalling by recruiting phosphatases such as SHP1 (SRC-homology-2-domain-containing protein tyrosine phosphatase 1).

### Ly49: regulation of NK-cell effector function

NK cells have essential direct and indirect roles in innate defence against infection and transformed cells. In response to diseased (as well as to normal) host cells, multiple distinct activation receptors induce the exocytosis of cytolytic granules and the production of immunoregulatory cytokines by NK cells. Inhibitory receptors, many of which are specific for MHC class I, counteract NK-cell activation. Target-cell lysis occurs when NK-cell activation exceeds inhibition signalling<sup>57</sup>. Consequently, inhibitory signals set the activation threshold required for NK-cell-mediated target-cell lysis.

Co-expression of Ly49A and H2-D<sup>d</sup> on the NK-cell membrane does not lead to constitutive ITIM phosphorylation (L. Scarpellino and W.H., unpublished observations) and there is no evidence that the functionality of these NK cells is impaired<sup>15</sup>, indicating that Ly49A *cis* interactions do not generate tonic inhibition signals. Rather, the H2-D<sup>d</sup> on the NK cells sequesters a significant fraction of Ly49A receptors, and this restricts the pool of Ly49A receptors that are available for functional interaction with MHC class I ligands on target cells. This is apparent at the site of NK-cell-target-cell contact, the so-called immunological synapse. when NK cells do not express H2-D<sup>d</sup> themselves, Ly49A is efficiently recruited to the NK-cell synapse by the H2-D<sup>d</sup> expressed on target cells<sup>34,58</sup>. By contrast, Ly49A accumulation is strongly reduced when NK cells express H2-D<sup>d</sup>, suggesting that only the initially accessible Ly49A receptors can be recruited to the NK-cell synapse and that *trans* H2-D<sup>d</sup> cannot compete with *cis* H2-D<sup>d</sup> for Ly49A binding. This restricts the inhibitory capacity of Ly49A in the context of H2-D<sup>d</sup> expression<sup>15,33</sup>.

#### Immunological synapse

A term derived from the similarities to the synapses that occur in the nervous system, it defines a region that can form at the cell surface between two cells of the immune system that are in close contact, such as the interaction between a T cell and a natural killer cell with an antigen-presenting cell and a target cell, respectively. This interface involves adhesion molecules, as well as antigen receptors and cytokine receptors.

The moderation of the inhibitory signalling capacity renders NK cells more useful, as the activation threshold required for target-cell lysis is lowered. As an example, stress-associated expression of NKG2D ligands by host cells enhances NK-cell activation signalling. This is sufficient to induce target-cell lysis when NK-cell inhibition is moderate (FIG. 3a) but it may be insufficient when NK-cell inhibition is too potent (FIG. 3b). Likewise, when the number of available inhibitory receptors is limited, even a minor reduction of MHC class I ligand expression by host cells is sufficient to abrogate NK-cell inhibition and consequently lead to target-cell lysis<sup>59</sup>. Thus, the restriction of inhibitory signalling by *cis* interaction considerably improves the sensitivity of NK cells to react to diseased host cells. Despite the predominance of sequestered Ly49A, the number of residual Ly49A receptors that can functionally interact in *trans* is sufficient to prevent auto-aggression of non-stressed host cells expressing MHC class I molecules at physiological levels<sup>33,60</sup>.

### LILRB2 and PIRB: regulators of mast-cell effector function

Mast cells have essential protective roles in IgE-mediated immune responses, such as host protection against parasites. Mast cells are activated by the high-affinity Fc receptor for IgE (FcεRI), when bound IgE is crosslinked by multivalent antigens. Mast-cell activation triggers mast-cell degranulation, releasing vasoactive amines, such as histamine, and induces the synthesis of pro-inflammatory molecules and cytokines. excessive or aberrant mast-cell activation is associated with allergic reactions (reviewed in REF. 61).

Similar to NK cells, mast-cell activation is counter-regulated by several distinct inhibitory receptors, including PIRB<sup>16,62</sup>. Indeed, PIRB engagement by MHC class I dampens mast-cell activation<sup>16</sup> (FIG. 3c). Cell mixing experiments have shown that mast-cell activation is not significantly attenuated when the PIRB-MHC class I interaction occurs exclusively in *trans*<sup>16</sup>. The inhibitory effect of PIRB on mast-cell activation therefore depends on MHC class I recognition in *cis* (FIG. 3d). even in the absence of deliberate mast-cell activation, PIRB is constitutively tyrosine phosphorylated and associated with SHP1 (REFs 16,<sup>63</sup>). Thus, in contrast to Ly49A, the PIRB-MHC class I *cis* interaction may generate cell-autonomous, tonic inhibitory signals (FIG. 3c). Tonic suppression seems to prevent 'spontaneous' mast-cell degranulation, which may occur by 'accidental' aggregation of FcεRI-bound IgE. MHC class I expression in *cis* might lead to PIRB aggregation and/or, according to the concept of co-ligation, mediate an association of PIRB with the activating receptor FcεRI.

The new findings regarding PIRB function in mast cells contrast with the role of LILRB1 and LILRB2 in NK cells and myeloid cells, respectively, in which receptor engagement by HLA ligands on other cells dampens cellular activation<sup>13,64</sup>. This provides evidence that the LILR and PIR family receptors can also functionally interact with MHC class I molecules in *trans*. The precise contribution of *cis* versus *trans* interactions involving LILRs and PIRs to inhibitory signalling therefore warrants further investigation. Similar to CD22 (see later), recognition of MHC class I by LILRs or PIRs in *cis* and/or in *trans* may depend on whether effector cells are activated by cell-bound ligands as opposed to soluble ligands.

In addition to MHC class I, LILRB1 recognizes the MHC-class-I-like molecule UL18 encoded by human cytomegalovirus (HCMV). *Cis* binding by LILRB2 raises the issue of whether UL18 interacts with LILRB1 on the surface of HCMV-infected cells. This could have a role for latent HCMV infection, which is established in myeloid lineage cells<sup>65</sup>, which often express LILRB1.

## Siglecs: CD22 regulation of B-cell activation

Similar to the two-way recognition of MHC class I ligands, sialic-acid modifications of glycoproteins or glycolipids are recognized in *cis* and *trans* by several Siglecs (sialic-acid-binding immunoglobulin-like lectins). Siglecs are type I membrane proteins with an N-terminal V-type immunoglobulin domain that binds sialic acids and variable numbers (1-16) of C-type immunoglobulin domains (reviewed in REF. <sup>66</sup>).

The most extensively characterized Siglec is CD22 (also known as Siglec-2), a negative regulator of B-cell receptor (BCR) signalling. CD22 is a seven-immunoglobulin domain receptor, which binds sialic acids linked to galactosidase (Sia α2-6Gal) typically found on N-linked glycans of glycoproteins. In B cells, CD22 is largely inaccessible to soluble, multivalent sialoside probes, owing to the interaction of CD22 in *cis* with sialic acids<sup>67,68</sup>. The crucial sialic-acid-modified *cis* ligand is unknown, even though CD22 can bind to membrane IgM, CD45 and CD22 itself. Access to the CD22 receptor is restored when sialic acids are removed by sialidase treatment<sup>67</sup>, or in mice lacking the sialyltransferase ST6GAL1 (REF. 68).

In the absence of deliberate antigen exposure, basal BCR signalling is increased in B cells lacking CD22, demonstrating that the primary function of CD22 is to dampen BCR signalling<sup>69</sup>. The direct and/or indirect association of CD22 with the BCR complex in *cis* therefore mediates tonic suppressive signals that prevent 'spontaneous' B-cell activation (FIG. 3e). On B-cell activation by soluble antigen, the CD22 ITIMs are phosphorylated by the BCR-associated SRC-family kinase LYN. The ensuing recruitment of SHP1 to CD22 dampens BCR signalling (FIG. 3f). CD22 variants that selectively lack sialic-acid-binding capacity mediate reduced SHP1 recruitment to CD22, and consequently increased BCR signalling<sup>69,70</sup>. Thus, direct or indirect association with the BCR complex in *cis* is essential for the inhibitory function



of CD22. This is in contrast to Ly49 receptors, where *cis* association reduces the inhibitory function.

In addition to soluble antigens, B-cell activation by cell-bound antigen is also reduced when the stimulating cell displays sialic-acid modifications<sup>71</sup>. So, despite the fact that CD22 is normally masked, CD22 *trans* ligands negatively regulate BCR signalling. Indeed, unlike Ly49A<sup>34</sup>, sialic-acid-containing *trans* ligands efficiently recruit CD22 to sites of cell-cell contact<sup>72</sup>, indicating that masking is not stable, and that CD22 can switch from a *cis*- to a *trans*-bound state (FIG. 3g). This may be of importance to prevent B-cell activation by cell-bound self antigens.

## Beyond the immune system

Receptors that can bind the equivalent ligand expressed in *cis* and *trans* via the same ligand-binding domain have also been found in biological situations other than the immune system. For example, ephrin receptors (Ephs) mediate cellular repulsion on interaction with ephrin ligands expressed by other cells. This permits the sorting of distinct cell types or directs the growth of axons. Recent data suggest that Eph activity is influenced by ephrins expressed in the plane of the same membrane<sup>73</sup>. Indeed, Ephs can bind ephrin ligands expressed in *cis* via the ligand-binding domain of the receptor<sup>74</sup>. Interestingly, *cis* interactions did not induce Eph tyrosine kinase activity; rather, signalling by Eph to mediate cellular repulsion was reduced<sup>74</sup>. Eph therefore provides the first example of an activating receptor that is subject to regulation by a ligand interaction in *cis*.

## Prospects and predictions

So far, immunoreceptors that can bind the same ligand in *cis* and *trans* are receptors with inhibitory function. The emerging theme is that *cis* interactions modify inhibitory signalling. Whereas *trans* interactions are always inhibitory, significant differences exist with regard to the precise role of *cis* interactions. *Cis* interactions are required for the inhibitory function of CD22 and probably also PIRB, whereas they reduce the overall inhibitory capacity of Ly49 receptors. Consequently, *cis* interactions can serve to increase or decrease the threshold at which cellular activation signalling produces a biological response.

Following the discovery of inhibitory MHC class I receptors on NK cells, a plethora of inhibitory immunoreceptors has been identified<sup>55</sup>. Many of these receptors show wide tissue distribution whereby ligands may be co-expressed with their receptors on the same cell. It therefore seems likely that the activity of additional inhibitory receptors and perhaps also activating receptors is controlled via *cis* interactions, a possibility that should be considered along with *trans* interactions in the study of immunoreceptors.

### DATABASES

**Entrez Gene:** <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene>

[CD22](#) | [CD94](#) | [Ly49A](#) | [NKG2D](#) | [PIRB](#)

**Protein Data Bank:** <http://www.rcsb.org/pdb/home/home.do>

[1A6Z](#) | [1MI5](#) | [1P4L](#) | [1P7Q](#) | [1QO3](#)

### FURTHER INFORMATION

**Werner Held's homepage:** <http://www.lau.licr.org/pages/RG-NKTCDG.htm>

**Roy Mariuzza's homepage:** <https://carb.umbi.umd.edu/user/mariuzza>

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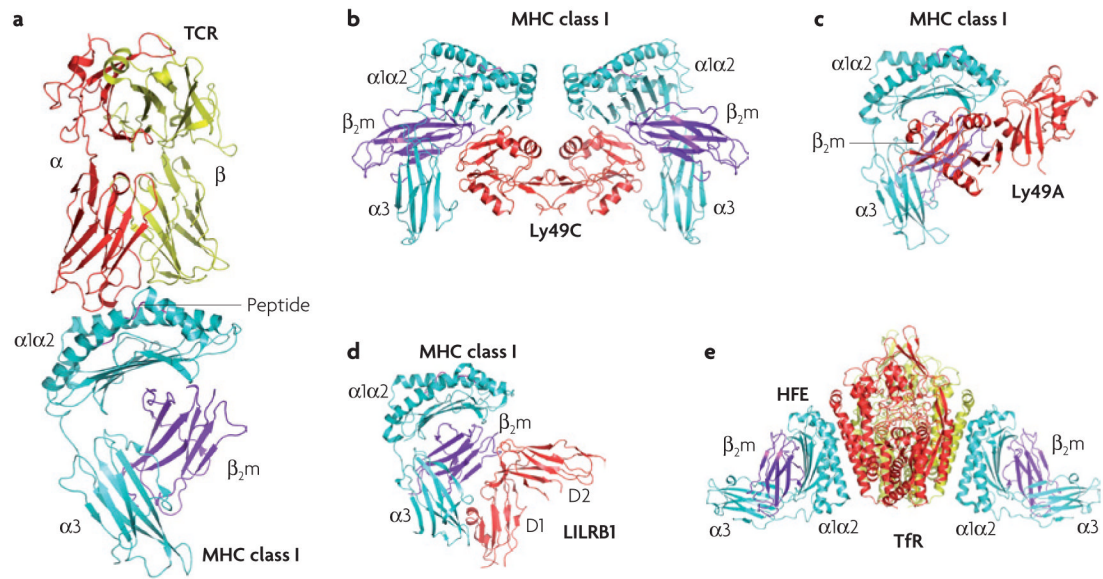
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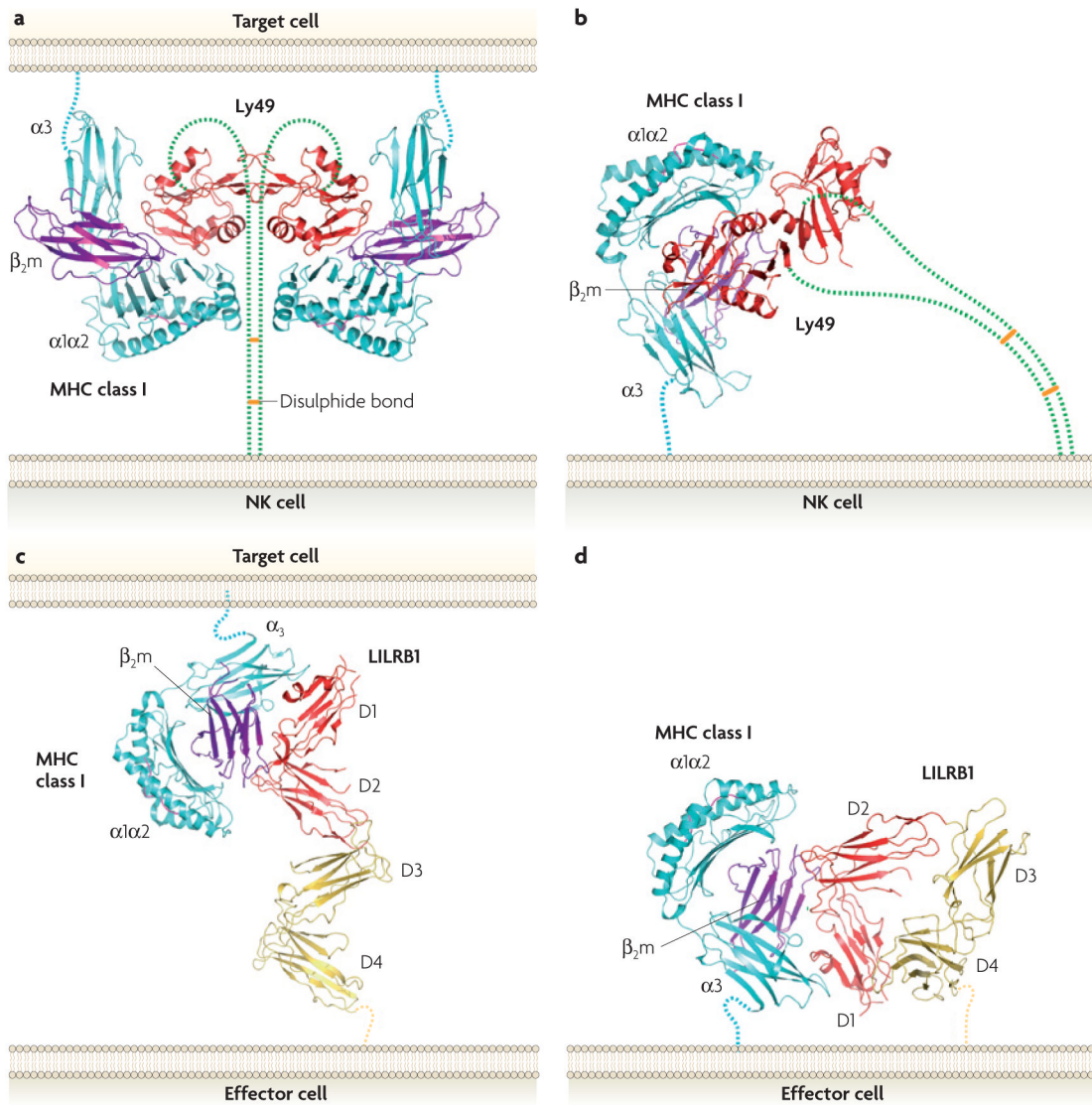
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### Figure 1. Structures of MHC class I receptors

Ribbon diagrams showing the crystal structures of several MHC class I receptors bound to MHC class I ligands. The  $\alpha 1$ ,  $\alpha 2$  and  $\alpha 3$  domains of the MHC class I heavy chain are shown in cyan,  $\beta_2$ -microglobulin ( $\beta_2m$ ) is shown in purple and the MHC-bound peptide is shown in pink. **a** | A typical T-cell receptor (TCR)-peptide-MHC-class-I complex is shown (protein data bank (PDB) ID: [1MI5](#)). The TCR  $\alpha$ - and  $\beta$ -chains are shown in red and yellow, respectively. **b** | The Ly49C-H2-K<sup>b</sup> complex is shown (PDB ID: [1P4L](#)). The two C-type lectin domains (CTLDS) of Ly49C (shown in red) bind two H2-K<sup>b</sup> molecules in a symmetrical arrangement. The Ly49C dimer is in the ‘open’ conformation. **c** | Representation of the Ly49A-H2-D<sup>d</sup> complex (PDB ID: [1QO3](#)). The two CTLDS of Ly49A (shown in red) bind a single H2-D<sup>d</sup> molecule in an asymmetrical manner. The Ly49A dimer is in the ‘closed’ conformation. **d** | Representation of the LILRB1 (leukocyte immunoglobulin-like receptor B1)-HLA-A2 complex (PDB ID: [1P7Q](#)). The D1 and D2 domains of LILRB1 are shown in red. **e** | The transferrin receptor (TfR)-haemochromatosis protein (HFE) complex is shown (PDB ID: [1A6Z](#)). The two subunits of the TfR dimer are shown in red and yellow. HFE, a non-classical MHC class I molecule, must assume a supine orientation on the cell membrane to bind TfR in *cis*. By contrast, the MHC class I molecules in (**a-d**) are in upright orientations.

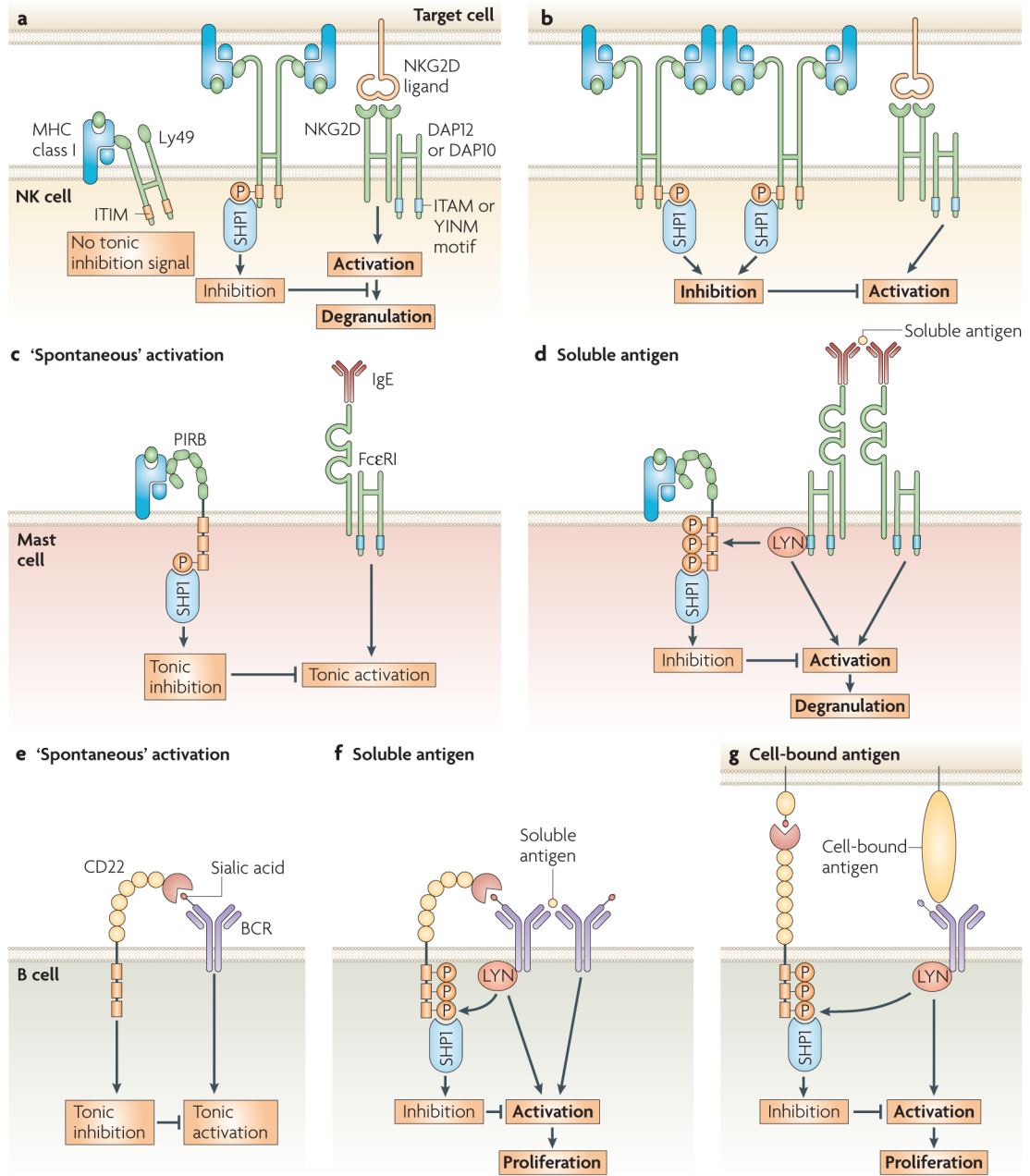


**Figure 2. Hypothetical models for *trans* and *cis* interactions of ly49 receptors and LILRs with MHC class I ligands**

**a** | *Trans* interaction of a Ly49 receptor with two MHC class I molecules, based on the crystal structure of the Ly49C-H2-K<sup>b</sup> complex<sup>26</sup>. The  $\alpha 1$ ,  $\alpha 2$  and  $\alpha 3$  domains of the MHC class I heavy chain are shown in cyan,  $\beta_2$ -microglobulin ( $\beta_2m$ ) is shown in purple and Ly49C is shown in red. The Ly49C homodimer on the natural killer (NK) cell binds two H2-K<sup>b</sup> molecules on the target cell. The Ly49C-H2-K<sup>b</sup> structure does not include the 70-amino-acid stalk regions that connect the Ly49C homodimer to the NK-cell membrane. The stalks are drawn arbitrarily here (in green), with horizontal orange bars to indicate disulphide bonds. To bind in *trans*, the stalks must adopt a back-folded conformation, as the N-termini of the Ly49C monomers point away from the NK-cell membrane (Ly49 receptors are type II transmembrane proteins). **b** | *Cis* interaction of Ly49 with MHC class I, based on the structure of the Ly49A-H2-D<sup>d</sup> complex<sup>27</sup>. The Ly49A homodimer binds one H2-D<sup>d</sup> molecule on the NK cell itself. In this case, the stalks may assume an extended conformation, as the N-termini of the Ly49A monomers point towards the NK cell. **c** | *Trans* interaction of a leukocyte immunoglobulin-like receptor (LILR) with MHC class I, based on the structure of the LILRB1-HLA-A2 complex<sup>40</sup>. The LILRB1-HLA-A2 structure includes the two N-terminal immunoglobulin-

like domains of LILRB1 (D1 and D2), but not its two C-terminal immunoglobulin-like domains. Domains 3 and 4 (D3 and D4), which link the ligand-binding domains (shown in red) to the surface of the effector cell, are represented by homology models (shown in yellow). **d** | *Cis* interaction of LILRB1 with MHC class I. To bind to HLA-A2 on the same cell, LILRB1 must bend back on itself, presumably at the connecting region between D2 and D3.





**Figure 3. Physiological role of *cis* and *trans* interactions of Ly49A, PIRB and CD22**

**a** | Stable association of Ly49 with H2-D<sup>d</sup> in the plane of the natural killer (NK)-cell membrane (in *cis*) reduces the pool of Ly49A receptors available to engage H2-D<sup>d</sup> on target cells (in *trans*). When NK-cell inhibition is restricted, further NK-cell activation, upon the recognition of the NKG2D (NK group 2, member D) ligand on stressed host cells, results in target-cell lysis. **b** | In the absence of Ly49A-H2-D<sup>d</sup> interactions in *cis*, all Ly49A receptors can interact with H2-D<sup>d</sup> in *trans*, resulting in strong NK-cell inhibition. Limited additional NK-cell activation may not suffice to induce lysis of stressed host cells. Ly49A association with H2-D<sup>d</sup> in *cis* decreases the NK-cell activation threshold. **c** | In the absence of deliberate mast-cell activation, paired immunoglobulin-like receptor B (PIRB) is constitutively phosphorylated and associated with MHC class I in the mast-cell membrane, suggesting that *cis* interactions

mediate tonic suppression of spontaneous mast-cell degranulation. **d** | Mast-cell activation by crosslinking IgE bound to FcεRI (high-affinity Fc receptor for IgE), increases PIRB phosphorylation and the recruitment of SHP1 (SRC-homology 2 (SH2)-domain-containing protein tyrosine phosphatase 1). Unlike Ly49A, the inhibitory function of PIRB depends on *cis* interaction. PIRB *cis* interaction increases the threshold for mast-cell activation. **e** | CD22 is directly and indirectly associated with the B-cell receptor (BCR) by binding sialic-acid-modified glycoproteins. CD22 *cis* association dampens basal BCR signalling and prevents 'spontaneous' B-cell activation. **f** | Following B-cell activation with soluble antigen, the association of CD22 with the BCR is needed for the inhibitory function of CD22. CD22 *cis* association increases the threshold for B-cell activation. **g** | CD22 can switch from a *cis*- to a *trans*-bound state on the encounter of sialic-acid ligand on opposing membranes. The engagement of CD22 in *trans* is inhibitory and increases the threshold for B-cell activation. ITAM, immunoreceptor tyrosine-based activation motif; ITIM, immunoreceptor tyrosine-based inhibitory motif.

**Table 1**  
Inhibitory receptors specific for MHC class I molecules and viral proteins

Receptor*	Species	Structure	Stalk length (amino acids)	Ligand	Binding site <sup>‡</sup>	Cis binding	Expression	Refs
<i>KIR family (CD158)</i> <sup>§</sup> KIR2DL1, KIR2DL2, KIR2DL3	Human	2 immunoglobulin-like domains	40	HLA-C allotypes	Top	No; ND <sup>//</sup>	Subsets of NK and effector T cells Mostly NK cells	
KIR2DL4	Human	2 immunoglobulin-like domains	40	HLA-G	ND	ND		
KIR3DL1	Human	3 immunoglobulin-like domains	40	HLA-B <sup>Bw4</sup>	Top?	ND	Subsets of NK and effector T cells	
KIR3DL2	Human	3 immunoglobulin-like domains	40	HLA-A3, HLA-A11	ND	ND	Subsets of NK and effector T cells	
<i>Ly49 family (KLRA)</i> Ly49A, Ly49C, Ly49F, Ly49G, Ly49I	Mouse	CTLD	72-76	Classical H2 molecules	Lateral	Yes	Subsets of NK and effector T cells	15, 21,25
Ly49I <sup>129</sup>	Mouse	CTLD	76	MCMV m157	ND	ND	Subsets of NK and effector T cells	75
Ly49B	Mouse	CTLD	78	Classical H2 molecules	ND	No	Myeloid cells	25,76
Ly49Q	Mouse	CTLD	77	H2-K	ND	yes	DC and myeloid cells	25,77
<i>LILR and PIR family (ILT, LIR, MIR, CD85)</i> <sup>//</sup> LILRB1 (LIR1, ILT2)	Human	4 immunoglobulin-like domains	53	Various HLA molecules	Lateral	ND	NK, T, B and myeloid cells	13
LILRB2 (LIR2, ILT4)	Human	4 immunoglobulin-like domains	42	HCMV UL18	Lateral	ND		37
PIRB	Mouse	6 immunoglobulin-like domains	42	Various HLA molecules	ND	Yes	B, myeloid and mast cells	16,64
CD94-NKG2 <sup>§</sup> CD94-NKG2A	Human	CTLD	29 (CD94); 19 (NKG2A)	Various H2 molecules	ND	Yes	B, myeloid and mast cells	14, 16,41
CD94-NKG2A (KLRD1-KLRC1)	Mouse	CTLD	29 (KLRD1); 26 (KLRC1)	Qa-1 <sup>b</sup>	Top	No <sup>//</sup>	Subset of NK and effector T cells	11
					Top		Subset of NK and effector T cells	12

\* Receptor families include inhibitory and stimulatory receptors. Alternative names for receptors are provided in parentheses.

<sup>‡</sup>Top:  $\alpha 1$  and  $\alpha 2$  domains of MHC class I heavy chain and bound peptide; lateral:  $\alpha 3$  domain and  $\beta 2$ -microglobulin (plus  $\alpha 2$  domain for Ly49).

<sup>§</sup>For a review, see REF. 44.

<sup>//</sup>Unpublished data.

<sup>//</sup>For a review, see REF. 39. CTLD, C-type lectin-like domain; DC, dendritic cell; HCMV, human cytomegalovirus; ILT, immunoglobulin-like transcript; KIR, killer-cell immunoglobulin-like receptor; LILR, leukocyte immunoglobulin-like receptor; MCMV, mouse cytomegalovirus; MIR, monocyte/macrophage immunoglobulin-like receptor; ND, not determined; NK, natural killer; NKG2, NK group 2; PIR, paired immunoglobulin-like receptor.